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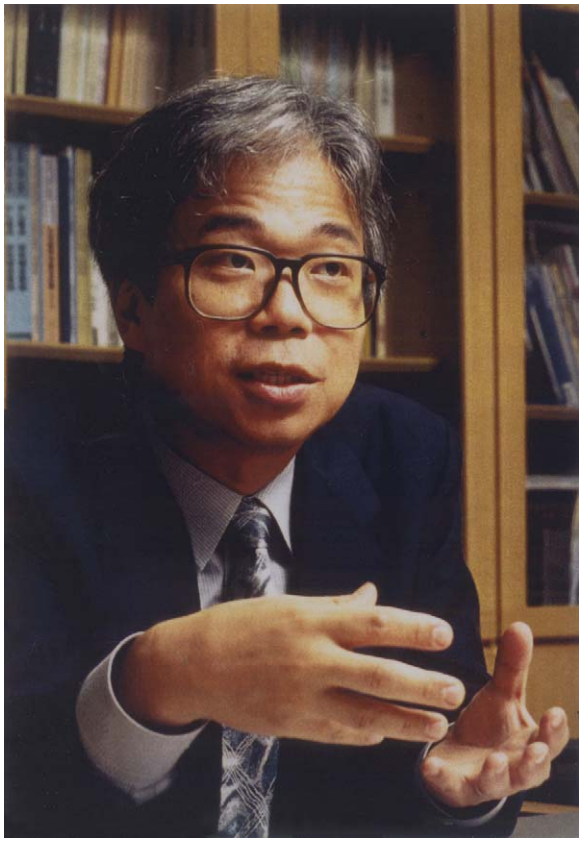
Obituary

In Memoriam: Shoichiro Tsukita (1953–2005)



Shoichiro Tsukita in his office in Kyoto University. Photo courtesy of Sachiko Tsukita.

Shoichiro Tsukita, Professor of Cell Biology at Kyoto University, passed away in Kyoto, Japan, on December 11, 2005, at the age of 52. He was an inspiring and energetic scientist and mentor and will be remembered with respect and affection by his colleagues and friends. Tsukita pioneered the use of the isolation of cell-cell junctions from tissues to define the molecular architecture of adherens and tight junctions, work that led to the discovery of a number of junction components including the claudin family of membrane proteins now known to be essential elements of tight junction structure.

Tsukita began his scientific career as an undergraduate student in the University of Tokyo in early 1970s, at a time when molecular biology in bacteria was maturing as a field of science. He initially hoped to explore brain function using molecular biology, and as a first step he chose to study brain morphology using the electron microscope. He visited an electron microscopist, Eichi Yamada, in the Tokyo University Faculty of Medicine, and Yamada's talks on histology impressed the young Tsukita deeply. Tsukita went on to learn electron microscopy techniques under the supervision of Harunori Ishikawa, but he soon came to the conclusion that the brain was far too complex to analyze in this way. During this period, he also met Sachiko, who became his wife and remained his colleague for the rest of his life.

In the course of his electron microscopy studies, Tsukita also became deeply fascinated by the elaborate design of cell structure, including the cytoskeleton. Working in Yamada's laboratory, Tsukita initiated research into the mechanism of axonal transport. He continued his work as a graduate student in the same laboratory, earning both his M.D. and his Ph.D. and becoming a lecturer in the department. Axonal transport involves anterograde (cell body to periphery) and retrograde (periphery to cell body) pathways. By applying local cooling instruments on mouse legs, Tsukita inhibited transport in both directions in situ and revealed that distinct sets of membranous structures are transported in each direction. As microtubules are required for each form of transport and microtubule plus ends are directed toward the periphery, his findings suggested that different types of microtubule-based motor proteins and adaptor proteins are involved in anterograde and retrograde transport, a pioneering conclusion in this field. He tried to identify a motor protein responsible for the transport for some time, but without success. Later, when kinesin, a microtubule plus end-directed motor protein, was identified in squid axon, I recall him commenting with regret that he might have had a chance to make that discovery if he had continued his work.

Tsukita had a wide range of interests in cell biology, which included the mechanisms of ciliary movement and muscle contraction. He studied both using electron microscopy combined with flash-freezing techniques that had been developed to a sophisticated level in his laboratory. He found that myosin heads in skeletal muscles rapidly frozen during contraction showed regular alignment, giving the impression that individual myosin heads do not change their apparent conformation during contraction, an observation that stood in stark contrast to the well-known lever-arm swinging model. Although he enjoyed these studies in the field of biophysics, Tsukita began to worry that he had begun to stray a bit too far from his original path.

Tsukita opened his own laboratory, with Sachiko, at the Tokyo Metropolitan Institute for Medical Sciences in 1986. He intended to make the most of his strong grounding in histology to elucidate mechanisms of cell-cell communication through structural analyses of epithelial cell junctions, and he decided to embark on a new project of isolating adherens junction structures from tissues. Adherens junctions are characterized by the presence of cadherin family adhesion molecules and the association of actin filaments. Tsukita chose to use rat liver, as cells in this tissue contain very little cytoskeleton except for that connected to the highly organized cell adhesion sites. His group developed a method for obtaining adherens junction fractions by modifying a protocol for the purification of bile canaliculi from the liver. The

idea was to generate monoclonal antibodies against each of the protein constituents of the adherens junction and then use those antibodies for protein localization and cDNA cloning, This approach was rooted in Tsukita's confidence in the idea that proteins involved in building a given structure must also have some role in the function of that structure. Tsukita saw that once its cDNA has been obtained, the function of a protein can be tested. His confidence was borne out when this strategy began to give rise to a series of breakthrough discoveries.

Tsukita moved to Okazaki National Institute for Physiological Sciences in 1990 and continued with his adherens junction work. During this period, his laboratory cloned a number of important molecules including α-catenin, an essential component of the cadherin/catenin complex; ZO-1, a PDZ domain-containing molecule that was originally thought to be the sole tight junction marker; and radixin, a founding member of the ERM family of proteins, which function as plasma membrane/actin filament crosslinkers. Following these discoveries, functional characterization began in earnest. Defects in these or related proteins are frequently tumorigenic, and, as the origin of the majority of tumors is epithelial cells, Tsukita was excited to think that his work might one day make significant contributions to basic cancer research. At this time, Shoichiro and Sachiko Tsukita had a son, who was still quite young. Every evening they picked him up at his nursery school on their way home from the lab, and the family would have dinner together at home. After their son fell asleep, Shoichiro and Sachiko would bring him back to the lab, where they transferred him to a bed in a corner of the room to sleep while they resumed their experiments.

In Okazaki, Tsukita's studies entered a new phase. He realized that adherens junction fractions from chicken liver also contained tight junction components, since the staining patterns of many tissues with one of the monoclonal antibodies was quite similar to that of tight junctions. Tight junctions are unique to the epithelial and endothelial cells that cover the outer and inner surfaces of the body, demarcating various cellular compartments. These junctions function as barriers that regulate solute diffusion through the intercellular space, making it possible to maintain a stable environment within the compartment. Although the mechanisms underlying tight junction function had been examined to some extent, mainly by physiological approaches, information about the molecules was very limited.

By the early 1990s, ZO-1 and cingulin had been identified as cytoplasmic proteins localized at tight junctions, but a tight junction integral membrane protein had yet to be discovered. Thus, when Tsukita's group identified occludin as the first membrane protein localized to tight junctions in 1993, it was a major achievement. Tight junctions appear as a set of anastomosing intramembranous particle strands when observed using freeze-fracture replica electron microscopy, and the distance between any pair of opposed membranes is zero. They found that occludin precisely localizes to the tight junction strands, and its putative four membrane-spanning segments made occludin unique among integral membrane proteins at cell-cell junctions. Stimulated by this discovery, Tsukita's lab set out to obtain a cDNA for mammalian occludin.

In 1994, Tsukita moved to the Kyoto University School of Medicine, and it was there that mouse occludin cDNA was finally cloned and occludin-deficient cells and mice were generated. However, contrary to Tsukita's expectations, occludin mutant cells contained well-developed networks of tight junctions strands, clearly indicating that something other than occludin must be responsible for the formation of tight junctions. Tsukita went back to his junction fractions, and on reexamining a fraction isolated from chicken liver, found a silver-stained band of $\sim$22 kDa with the occludin band. The peptide sequences of the band led to the isolation of mouse cDNAs corresponding to a pair of proteins that localized at tight junction strands; these were designated claudin-1 and -2 in 1998. The group found that when either claudin-1 or -2 was introduced into L fibroblasts lacking tight junctions, well-developed networks of strands formed containing claudins. At last, Tsukita had found the fundamental essential component in the formation of tight junctions: claudins. Over the years, two competing models had been proposed to explain the molecular architecture of tight junction strands. One model posited that integral membrane proteins polymerize linearly within lipid bilayers and form strands, whereas an alternate theory suggests that lipid structures are responsible for the process. The identification of the claudins strongly supported the idea that integral membrane proteins play an important role in strand formation.

Claudins are now known to constitute a multigene family, and several members show tissue-specific expression patterns. Tsukita aimed to generate lines of mice deficient for each claudin systematically, and studies by his and other groups on knockout mice and human disease have revealed that claudins are key molecules in regulation of the barrier function of tight junctions throughout the body. The presence and functional importance of tight junctions in the epidermis were revealed by claudin-1-deficient mice, while claudin-5 was shown to contribute to the function of blood-brain barrier through its activity in the tight junctions linking brain endothelial cells. Claudin-11 is specifically expressed in the cochlea, and mice deficient for this gene suffer from deafness. Claudin-19 is expressed at peripheral nerves, localizes at tight junctions of Schwann cells, and is involved in the saltatory conduction of axon potentials. Mutations in claudin-16/paracellin-1 cause hereditary hypomagnesemia. Biochemical interactions between occludin/claudin and ZO-1, -2, -3, and other proteins were also examined in an effort to develop a better understanding of the mechanisms regulating tight junctions from the cytoplasmic side. When confronted with this wealth of functional diversity, Tsukita developed an interest in algorithms about combination of claudin molecules and the dynamics of tight junction strands as well.

Finding claudins was a major discovery, but, not surprisingly, it left Tsukita with yet more questions to solve. How can occludin and claudin polymerize linearly within lipid bilayers? By what mechanism do claudins regulate the paracellular transport of substances at tight junctions? What allows tight junction strands to restrict the lateral diffusion of lipids only in the outer leaflet of the plasma membrane? How do tight junction strands form selectively at the most apical region of the lateral

membranes in polarized epithelial cells? What role do claudins play in pathological conditions? Sadly, as he entered into this veritable treasure trove of questions awaiting solution, Tsukita was diagnosed with cancer.

Tsukita was always thinking about science and he genuinely enjoyed scientific discussions. He was fond of building up fascinating hypotheses, "Tsukita-san's great hypotheses," as he'd jokingly call them, but he would be the first to admit that not all of them were necessarily well-founded. His enthusiasm for science and the way that the atmosphere in his lab encouraged everyone to talk freely about their ideas attracted many graduate students. Members of the lab could be relied upon to critique Tsukita's speculations, and Sachiko, too, would sometimes gently suggest to him that he should not fall too deeply in love with his own ideas. To his credit, Tsukita was always grateful for criticism. He thought that presentation of one's work is an important part of the scientific process, and he enjoyed it very much. He, himself, made most of the slides used in presentations by members of his lab. Although he was a brilliant scientist, Tsukita never aspired to take on the airs of greatness and never hesitated to reveal himself as just another ordinary person. Scientific discussions were often lubricated by a bottle of *sake*, and Tuskita very much liked joking and engaging in light conversations as well. When he was in Okazaki, he became an avid tennis player and always selected the best player in his lab as a doubles partner. Tsukita was concerned about his physical condition and tried dieting. During his first attempt, he made a graph of his weight and showed its daily decrease to lab members. He lost 10 kg within a short period and began to pride himself on his strength of will; the extra pounds, however, had other ideas, and were back within a year. Undaunted, he made a number of additional forays into dieting, and his weight continued to fluctuate. Outside the lab, Tsukita was also an ardent baseball fan, and one of his fondest memories was a dinner with Japan's most famous player, Shigeo Nagashima. He also liked skiing, traveling, and driving with his family.

After his pancreatic cancer was diagnosed, Tsukita lived for another year and two months. Only his family and a few others knew of his condition. I cannot imagine what he felt and thought during those months, as he underwent treatments intended to extend his life to enable him to be with his family for as long as possible. Throughout, Tsukita continued his work on his research. Tricellulin, a newly characterized integral membrane protein responsible for forming junctions at contact points where three cells meet, was identified during this period. Always conscientious, he left both private words for his family and a book on his work for students and young scientists. He was sincerely grateful to everyone he came into contact with professionally or personally for the support they gave him. Although at the time of his death Tsukita was a renowned professor in charge of a big laboratory, the image of him as a young investigator in front of an electron microscope, formulating elaborate plans for obtaining revelatory samples, is the one I carry with me. I am sure that he will be remembered most by those who knew him for having shown how exciting a life lived in science can be.

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